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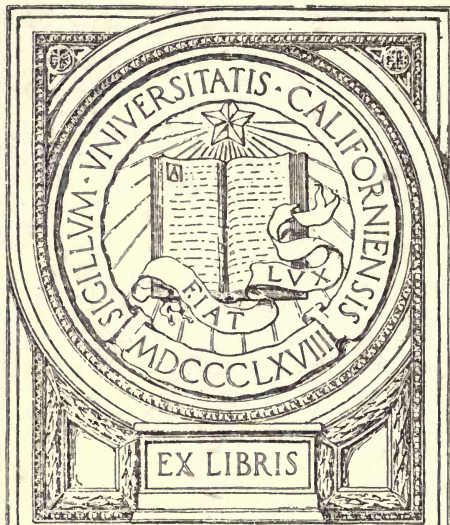


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EXCHANGE



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Experiments on Vitamin A

By

MARTHA KRAMER

EXCHANGE

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DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy in the Faculty of
Pure Science, Columbia University in the
City of New York



NEW YORK CITY

1922

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ABSTRACT

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ACKNOWLEDGMENT.

THE author is indebted to Professor H. C. Sherman for having suggested the problem and for advice and encouragement received throughout the work.

EXCHANGE

EXPERIMENTS ON VITAMIN A

I. INTRODUCTORY

The existence of the substance now known as vitamin A was established independently by McCollum and Davis (7) and by Osborne and Mendel (8, 9) through experiments in which it was found that animals on food mixtures alike in all other respects would grow or not according as the fat in the food mixture were butterfat or lard. This was shown to be due not to any differences between the actual fats, but to the presence in the butter of a fat-soluble growth-promoting substance.

As the vitamin was first noticed by the physiological effects of its absence from the diet and the changes produced when it was re-introduced, so it is now studied experimentally. The effects of its absence are failure to grow, the appearance of certain conditions of disease, and death if the experiment is sufficiently prolonged. The albino rat is the most suitable experimental animal for this work, a small animal well adapted to laboratory experiments and sensitive to a lack of vitamin A. A diet free of vitamin A but adequate in all other respects must be used. The substance whose richness in the vitamin is being tested may be incorporated in the food or it may be fed separately in measured amounts daily. The rats may receive the vitamin-containing food from the beginning of the experiment, in which case the measure of the vitamin A content is the protection the rat receives against the appearance of vitamin A deficiency symptoms, and also the rate of growth which the animal makes. These are usually called protective experiments. The rat may be fed on vitamin A free food for a long time, until he becomes quite sick and has very sore eyes; the material to be tested for vitamin A may then be supplied and its curative power demonstrated. Mid-way between curative and protective experiments is another type. Rats have in their bodies stores of vitamin A; they continue to grow for some time after being placed on vitamin A free food. The rats may be fed on vitamin A free food until their body store of the vitamin has been reduced and, at the point where the weight curves start to bend down, but before signs of sickness or disease have really appeared, feeding of the material containing vitamin A may be started.

For any of these experiments, the size of the dose to be given is of importance. Zilva and Miura (16) try to give only enough of the vitamin A containing food to cause slight growth after growth has been inhibited. This slight bending up of the weight curve is their standard for comparison. Drummond (1, 2) substitutes the food to be tested for part of the vitamin A free diet and takes the rate of growth of the rat—in comparison with other cases—as roughly proportional to the amount of vitamin A which has been supplied by the added material. For these or other types of work meant to be quan-

titative, doses must be sufficiently low that lesser doses would certainly give lesser growth. Comparisons should be made with controls of two sorts, (a) on the food free of vitamin A, and (b) on food otherwise the same but with enough vitamin A added to give good growth.

The exact methods for the experimental work on vitamin A vary with different laboratories and have not been standardized. In the first place, the rats used should measure up to some sort of standard for comparison. Such factors as age and weight, as well as previous history, should be taken into consideration. Greater differences exist in young rats than are usually taken into consideration in vitamin A experiments. The vitamin A free food used as the basis of the experiments varies with different laboratories. Young rats particularly are very sensitive to inadequacies in their diet. The vitamin A free food should be proven free of vitamin A and also proven adequate in all other respects, i.e., adequate for growth and reproduction when vitamin A is added. These points with regard to the standardization of the rat and also of the vitamin A free food used offer opportunity for investigation, some of which has been done and will be described in this paper.

Another method for the measurement of vitamin A which has not previously been used is the survival of older rats upon vitamin A free food, in which case the survival periods may be used as comparative measures of the vitamin A storage from the previous diets. The property of promoting the storage of vitamin A may not necessarily run parallel quantitatively with the vitamin A content, but for practical purposes of human nutrition, for which supposedly most vitamin A measurements would be made, it would be the point of first importance. The problem of storage of the vitamin is also related to the other methods of measurement, for which reason the previous diets of the young experimental rats must be taken into consideration. The method and results of many vitamin A storage determinations will be given.

II. STORAGE OF VITAMIN A IN THE BODY.

METHOD.

An important question concerning vitamin A is how and to what extent it is stored in the animal body. Grown rats are able to live for a long time on food deficient in vitamin A; it was desired to study the survival periods with relation to the previous histories of the rats.

An unusually good opportunity for these survival experiments was offered in this laboratory, since large numbers of rats were being fed on simple and easily comparable diets, in connection with other experiments. Many of the diets were fed extensively and through many generations. On account of the large number of rats on these laboratory diets, the results of growth on the diets were well understood. For the survival experiments, then, rats could always be

taken which were making normal growth for their diet. A great many very well matched cases were obtained, so that, in addition to important average results, other good comparisons are presented. Where possible, two or more rats of the same original litter were obtained, as checks on each other.

Since large quantities of the vitamin A free food were to be used, the food had to be of a sort easily prepared. Flour, known to be free of vitamin A and also known to contain proteins sufficient for the maintenance of the rat for a very long time, was used as the basis of the foods. Gold Medal Flour was used throughout the experiments for the sake of uniformity. Flour contains vitamin B in more than sufficient concentration for rats, so that only mineral supplements were added. The comparative survival periods of the rats from different laboratory diets were then taken as a measure of the comparative efficiency of those diets in promoting storage of vitamin A. The following results show that survival periods on any of these flour diets may be compared for measuring the vitamin A value of the previous diets, if the rats used are grown at the beginning of the experiment.

Rats Nos. 1767, 1768, 1769 and 1770 were healthy males, born August 27, 1920. They were raised on Diet 13A ($\frac{1}{3}$ whole milk, $\frac{2}{3}$ whole wheat, 2% NaCl* with fresh string beans ad libitum) and continued on this diet until January 4, 1921 when they were 130 days old. The four rats had grown well and so uniformly that they were very well matched for the survival experiment.

Rat No. 1767 was put on Diet 23, consisting of	
Patent flour	100
NaCl	2
Rat No. 1768 was put on Diet 83, consisting of	
Patent flour	95
NaCl	2
Calcium Lactate, $\text{Ca}(\text{C}_3\text{H}_5\text{O}_3)_2 + 5\text{H}_2\text{O}$	3
Rat No. 1769 was put on Diet 84, consisting of	
Patent flour	95.0
NaCl	2.0
Calcium Lactate, $\text{Ca}(\text{C}_3\text{H}_5\text{O}_3)_2 + 5\text{H}_2\text{O}$	2.9
Ferric Citrate, $\text{Fe}_2(\text{C}_6\text{H}_5\text{O}_7)_2 + 6\text{H}_2\text{O}$	0.1
Rat No. 1770 was put on Diet 85, consisting of	
Patent flour	95.0
NaCl	2.0
Calcium Lactate, $\text{Ca}(\text{C}_3\text{H}_5\text{O}_3)_2 + 5\text{H}_2\text{O}$	2.5
Ferric Citrate, $\text{Fe}_2(\text{C}_6\text{H}_5\text{O}_7)_2 + 6\text{H}_2\text{O}$	0.1
K_2HPO_4	0.4

* The expression "2% NaCl," as used in this dissertation, means sodium chloride to the extent of 2% of the weight of wheat, flour or starch in the dry food mixture.

At first, all the rats lost weight rapidly. Subsequently, their weight curves fluctuated in various ways, these fluctuations being more or less closely parallel by variations in the food consumption. Perhaps the great initial losses of weight were due to dissatisfaction with the foods, which were much less desirable than Diet 13A.

The rat receiving calcium (Diet 83) did not do appreciably better than the rat receiving only flour and salt (Diet 23). On the other hand, the two rats receiving additional iron soon increased their food intake and attained as high body weights as they had previously reached, which they were able to maintain for a number of weeks. The rat receiving additional phosphorus (Diet 85) showed about the same weight curve as the one without it (Diet 84).

After death, calcium determinations were made of each rat.

Rat	Calcium content	On basis of Initial weight		On basis of Max. wt.	
		Initial wt.	Normal Ca. for this weight	Max. wt.	Per Cent. Calcium
1767	1.817	255	2.601	255	0.680
1768	2.781	280	2.968	280	0.983
1769	2.776	257	2.621	290	0.957
1770	3.719	280	2.968	302	1.231

Thanks are due Miss F. L. MacLeod and Miss E. McCollum for the quantitative determinations of calcium.

As the rats were well matched at the beginning of the experiment on vitamin A free diets, presumably their body stores of calcium were about the same and normal for their weights. These amounts are given (13). The rat on flour and salt alone, No. 1767, evidently used a large part of his body store of calcium. No. 1768 and No. 1769 died with about the same amount of calcium that they had when put on the deficient diets. They both received liberal amounts of calcium in their diets, but apparently stored little or none of it. No. 1769, because of the iron in his food, grew and maintained a higher body weight than No. 1768. He may have stored a little calcium at this time. No. 1770 stored much calcium, undoubtedly because of the phosphorus added to his food. In spite of these large differences in calcium metabolism on the vitamin A free diets, the survival periods were practically the same; the order of survival did not follow the order of increasing mineral adequacy of the diets. Since the four rats had about the same store of vitamin A in their bodies at the beginning of the experiment and since they lived about the same length of time, their survival periods on any of the four diets may be taken as comparative measures of their storage of vitamin A. Figure 1 shows the weight curves of the four rats just described.

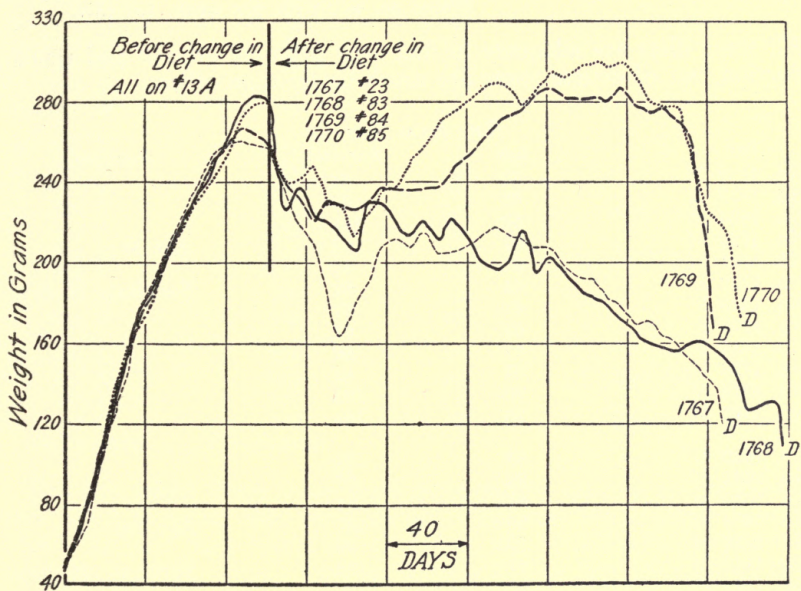


Figure 1. Showing uniformity of survival period as measure of previously stored vitamin, notwithstanding differences in mineral content of diet.

INFLUENCE OF PREVIOUS DIET AND OF AGE

Standard laboratory diets from which rats were drawn:

Diet	Whole Milk Powder	Whole Wheat	NaCl
A (16)	1/6	5/6	2% of whole
B (13)	1/3	2/3	wheat in all
			diets.
B (90)	—	2/3	+ 3/12 skim milk powder
			+ 1/12 butter fat
D (80)	2/3	1/3	
13A	13	+ fresh string beans ad libitum	
13M	13	+ fresh lean beef steak, at the rate of 60	
		grams per adult rat per week, fed raw.	

All these diets are adequate in that they promote growth and reproduction, but of course some are much better than others.

<i>Rats from Diet 16</i>			
Rat	Age	Survival	Averages
No. 1832 M	53	64	
No. 1833 M	53	66	
No. 1834 M	53	144	
No. 1720 M	61	62	Age 61
No. 1721 M	61	62	
No. 1722 M	61	61	Survival 76 days
No. 1723 F	61	61	
No. 1724 F	61	61	
No. 1725 F	61	62	
No. 1726 F	61	61	
No. 1376 M	70	106	
No. 1377 M	70	104	
No. 1630 M	84	73	
No. 1631 M	84	77	
No. 1632 M	84	67	
No. 1226 M	90	104	Age 100
No. 1228 M	90	89	
No. 1145 M	97	110	Survival 94 days
No. 1146 F	97	130	
No. 1147 F	97	101	
No. 1148 F	97	115	
No. 1149 F	97	69	
No. 1008 M	120	105	
No. 1009 M	120	106	
No. 1010 M	120	95	
No. 1011 M	120	72	
No. 655 M	159	89	Age 219
No. 661 M	196	92	
No. 609 M	208	108	Survival 89 days
No. 437 M	231	81	
No. 326 M	301	77	
M = male.		F = female.	

		<i>Rats from Diet 13</i>		Averages
Rat		Age	Survival	
No. 1471 M		60	116	
No. 1472 M		60	139	
No. 1473 M		60	103	
No. 1474 F		60	129	
No. 1475 F		60	136	
No. 1369 M		71	147	Age 78
No. 1370 M		71	130	
No. 1582 M		84	117	Survival 124 days
No. 1583 M		84	115	
No. 1584 M		84	78	
No. 1453 M		88	132	
No. 1454 M		88	120	
No. 1455 M		88	118	
No. 965 M		94	144	
No. 966 M		94	148	
No. 967 M		94	106	
No. 844 M		111	153	
No. 804 M		120	136	
No. 801 M		120	169	
No. 808 F		120	208	Age 132
No. 807 F		128	138	
No. 882 M		140	139	Survival 157 days
No. 810 F		142	150	
No. 685 M		152	133	
No. 834 F		156	184	
No. 424 M		238	105	
No. 427 M		238	185	Age 252
No. 498 F		265	112	
No. 499 F		265	95	Survival 124 days

M = male.

F = female.

The rats taken from these diets were placed on one of the flour diets, Diet 85 being used most of all. The rats were weighed and records kept of their food intake. Notes were made of the appearance of conditions which develop because of the lack of vitamin A. Eye disease is usually associated with the lack of vitamin A; many of these rats developed very bad eyes, including swelling of the eyelids, which were also often bloody and sticky, with inflammation of the cornea and sometimes loss of sight. Some rats, however, died before the eyes got into very bad condition. Sometime before death, nervousness, trembling and a stilted manner of moving the hind feet were always noticed, as well as increasing shabbiness in general appearance. Other abnormalities sometimes set in, as diarrhea. In all cases of survival periods cited, the lack of vitamin A was primarily the cause of death.

Rats from Diet 13A

Rat	Age	Survival	Averages
No. 985 M	89	176	Age 89
No. 986 M	89	155	
No. 987 M	89	130	Survival 154 days
No. 811 F	120	261	
No. 802 M	120	200	Age 126
No. 803 M	120	219	
No. 1767 M	130	227	Survival 231 days
No. 1768 M	130	256	
No. 1769 M	130	224	
No. 1770 M	130	237	
No. 835 F	130	225	
No. 892 F	136	139	
No. 885 M	136	218	Age 150
No. 888 M	136	217	
No. 832 F	141	291	Survival 249 days
No. 889 F	151	213	
No. 768 F	157	220	
No. 769 F	157	285	
No. 713 F	180	380	
No. 767 F	157	281	

M = male. F = female.

Comparisons of the survival periods of rats of different ages from the various diets were made. Since there was no greater variation between males and females than between members of the same sex, they were averaged together, as shown on the tables. The method used on the tables is as follows: There were eight rats from 120 to 130 days of age taken from Diet 13A. The average was 126 days old and the average survival period of the eight rats was 231 days. It could then be said that a rat 126 days old from Diet 13A should live about 231 days on one of the vitamin A free flour diets. In all cases, averages were sought which would be as representative as possible. The following figures were obtained from the data tabulated on pages 8, 9 and 10:

Rats from Diet	13			13A			16		
Age	78	132	252	89	126	150	61	100	219
Survived	124	157	124	154	231	249	76	94	89

Comparisons may be made in various ways. The curves in Figure 2 show the relation to previous diet, in the case of twin brothers reared from weaning time on diets A and B respectively and finally placed at the same age upon the same diet, Figure 2, deficient in vitamin A. It will be seen that the one from Diet B lived nearly twice as long on this food as the one from Diet A. Age and weight being constant, we find the effect of diet often clearly marked, as for instance:

Diet 13 rats 132 days old, initial wt. 246 gms., survived 157 days
Diet 13A rats 126 days old, initial wt. 257 gms., survived 231 days

Greater Storage of Vitamin "A" on Diet containing Higher Proportion of Milk

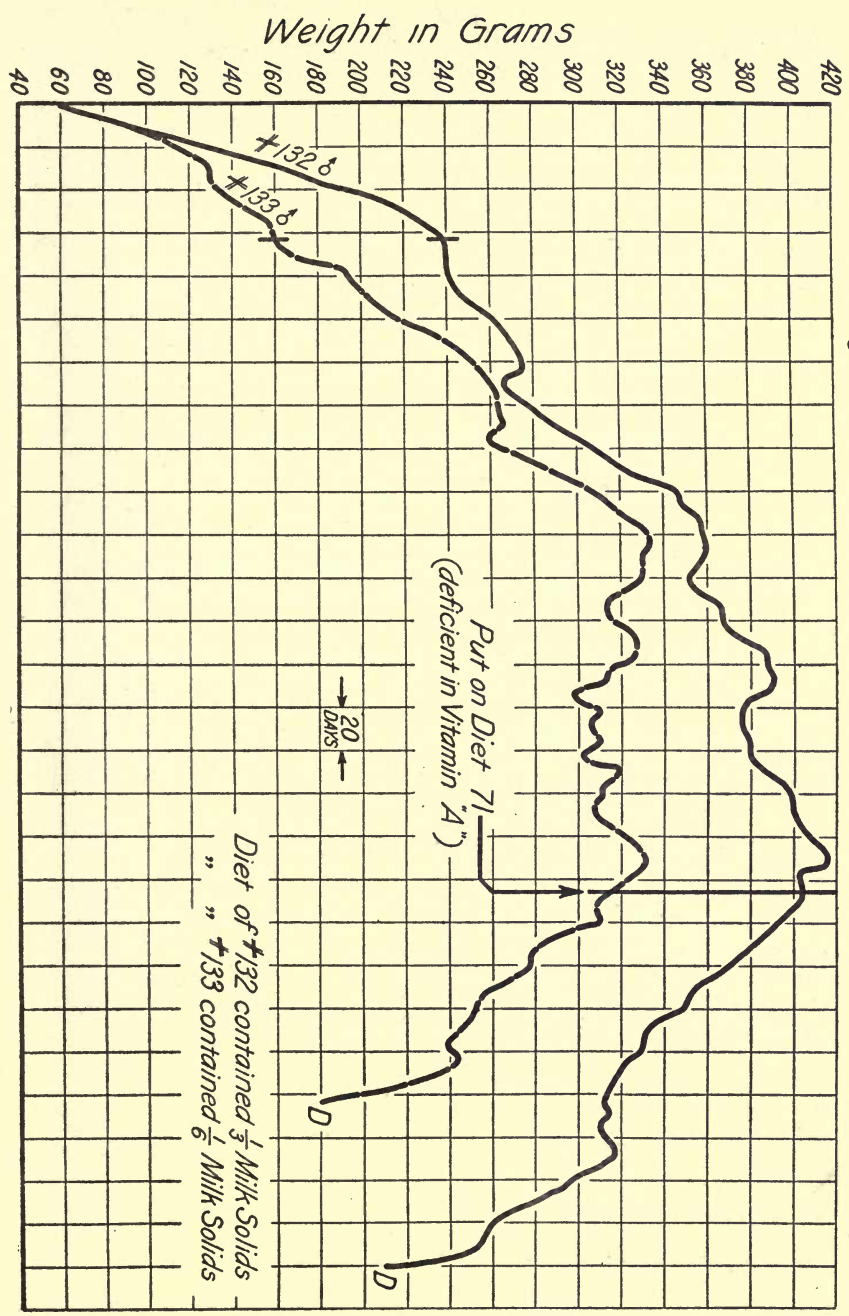


Figure 2. Influence of diet upon storage of vitamin A in the body.

III. WORK ON A VITAMIN A-FREE OTHERWISE ADEQUATE DIET.

BASIS OF DIETS

For the studies contemplated, it was necessary to have a thoroughly tested and reliable diet, free of vitamin A but adequate in all other respects. It was desirable also that this diet be as simple as possible and easily prepared in a uniform manner.

The diet proposed was:

Patent flour	82%
Meat residue	10
Salt mixture	4
Brewery yeast	4
	100%

The salt mixture was prepared according to Osborne and Mendel. (10, p. 572). In a few cases it was further enriched with iron and calcium but without evidence of any difference in results. Patent flour does not contain measurable amounts of vitamin A, according to results obtained by comparative feedings of flour and corn starch. (14) For the sake of uniformity, Gold Medal Flour was used, as has been the practice in this laboratory. Dry brewery yeast does not contain enough vitamin A to give noticeable results, even when fed as 42.5% of the diet (11). The meat residue was, then, the only material to be freed of vitamin A. If the vitamin could be removed from the meat residue easily and satisfactorily, it was thought that this would make a good diet for the experiments. The proteins of the flour, furnished at a rather high level, would be supplemented by the protein of the meat. Mineral constituents would be furnished in the salt mixture. Vitamin B, so necessary to rats would be furnished in sufficiently large quantities by the brewery yeast, supplementing the amount already in the flour. Vitamin C was neglected, as it does not seem to be of much importance to the rat (11).

The meat residue used was obtained from Valentines' Meat Juice Company, Richmond, Virginia. The product was in small dry lumps, the result of prolonged extraction with hot water. The meat residue was ground fine before using. Osborne and Mendel have recommended the use of meat residue as a source of good quality proteins for an A-free diet. Casein has been much used for this purpose, both in this country and in England. Commercial casein, granular in texture, contains more or less vitamin A which is rather difficult to remove completely. Osborne and Mendel suggested that possibly the solvents used did not penetrate the hard granular particles. The meat residue, on the other hand, has more of a fibrous texture and might be more readily and thoroughly penetrated by solvents used. Various methods for removing or destroying the vitamin A were tried.

DRY HEATING TO REMOVE VITAMIN A

Following suggestions obtained from the writings of Hopkins and Drummond (5, 2, 3) it was desired to test the effects of the dry heat on the meat residue, to see if vitamin A would be destroyed. The finely ground meat residue was heated in 100 gram portions in a flat granite pan in a hot air oven. This oven had a circulation of air not possible in an electric oven and was kept as nearly as possible at 105°C. The meat was stirred and raked over a number of times—method of Hopkins—during the heating, and since the layer of meat residue used was only about one centimeter thick and the material was quite loose in texture, the maximum of oxidation was provided for.

Meat Residue Heated 24 Hours.—The heated food was mixed and one-third of it was extracted by boiling three successive times with 95% alcohol for one hour and filtering under suction. The diet made up with the meat heated 24 hours was called 97-23 and that with the extracted meat residue was called 97-23-extracted. The diets were then fed as follows:

97-23, to get growth curves and survival periods with meat heated for 24 hours.

97-23-extracted, to see if any vitamin A escaped destruction by heat but was removed by the alcohol extraction.

97-23 + 10% butter fat, to see if 97-23 is adequate in all other respects, in which case normal growth would here be obtained.

Twelve animals were used in this way, the rats in each set being comparable and well matched with the rats in the other sets. Comparison of the rats on 97-23 and 97-23-extracted lead to the conclusion that no more vitamin A was removed by the extraction with alcohol. Periods of survival of comparable animals, in days, are as follows:

On 97-23	51	56	63	62
On 97-23-extracted	65	75	76	63

On the other hand the control animals receiving butter fat in their diet grew at much less than normal rate. No young were born until the rats had been on the diet for 25 weeks, which is very late for first breeding. This method of eliminating the vitamin from the meat residue is then successful in that vitamin A is eliminated but is not satisfactory in that the food cannot be said to be adequate in all other respects.

Meat Residue Heated 12 Hours.—The work was repeated with a

shorter period of heating. The diets were made up in the same way and numbered:

98-29 with meat heated 12 hours

98-30 with meat heated 12 hours and extracted with alcohol

98-29 + 10% butter fat.

It would seem that the heating was not sufficient to destroy the vitamin A for the rats on the food extracted died first. Periods of survival of comparable animals, in days, are as follows:

Diet 98-29 (meat heated 12 hours) 65 58

Diet 98-30 (meat heated and extracted) 54 44

The rats receiving butter fat grew at less than normal rate, although perhaps slightly better than in the case of the 24 hour heated food.

No more work has been started with dry heat in the presence of air as a method of eliminating vitamin A from the meat residue, because of the necessity of obtaining a diet adequate in all other respects. Since even with 10% of butter fat, rats receiving the 24 hour heated meat residue did not grow normally—and to a lesser extent the rats receiving 12 hour heated meat were not up to normal—the heating seems to damage the meat residue in some way besides destroying vitamin A (completely in 24 hours and partially in 12 hours). Knowing its limitations, foods prepared by dry heating for 24 hours might be used in cases where large numbers of animals were to be fed and where this method could be used easily and cheaply.

METHODS OF EXTRACTION TO REMOVE VITAMIN A

Three different methods of extraction with fat solvents were tried on the finely ground meat residue, to remove the fat and with it presumably all vitamin A. In each case the meat after treatment was made up into diets for the rats with flour, yeast and salt mixture in the same proportions as in previous experiments, flour 82%, meat residue 10%, brewery yeast 4% and salt mixture 4%.

The first method was similar to that of Osborne and Mendel (11) as used by Munsell (14). Twice the weight of the meat residue, of 95% alcohol was boiled for one hour with the meat under a reflux condenser, after which the hot alcohol was rapidly filtered off by suction. This process was carried out three times, 200 gram portions of the meat being used, after which the meat residue was dried and made into Diet 97-33.

The second method was a continuous extraction with hot 95% alcohol by means of an extractor which is essentially a modified coffee percolator. The hollow lid was arranged as a condenser. The meat residue was put into the container for coffee grounds. The long central tube of the percolator was discarded. Only alcohol vapor and condensed alcohol comes in contact with the meat which keeps hot. Fat-laden alcohol drops from the meat into the reservoir. This apparatus was used previously by M. Koch, the principle being the same as that of the Koch (6) extractor. The extractor was run on

a steam bath, so regulated that alcohol dropped steadily and rapidly from the condenser on to the meat residue. The last hour of extraction was always with a fresh portion of alcohol, so that the lower surface of the strainer, against which fat-laden alcohol splashed during vigorous boiling, might be thoroughly rinsed of fat. From the experience of Miss Koch and from our work it would seem that 12 hours were more than sufficient to remove all substances soluble in hot alcohol or in the fat. The 12 hour extracted product is the least, however, which has been used in these feeding experiments; the meat residue so treated was dried and made into Diet 97-34.

Acetone was used, cold, for removing the fat from the meat residue in the third method of extraction. Twice the number of cubic centimeters of acetone, as the weight of the meat in grams, was poured over the meat, in a funnel, the acetone being used in three portions. The acetone was allowed to run through slowly, the last portions being removed by suction and being only slightly straw colored. The meat residue was then extracted twice by boiling alcohol, as in the first method. Diet 97-35 was prepared from it.

A number of comparisons were started, with lots of rats on the three diets 97-33, 97-34, and 97-35 as evenly matched as possible as to previous history, age, weight and sex. Two sets were started in which there were controls receiving vitamin A in addition to the test diets, to see if the diets were adequate in all other respects. At this time the question had risen in the laboratory, whether the addition of 10% butter fat insured the presence of sufficient vitamin A for rats. The butter fat is most likely quite sufficient in this quantity, but because of the season of the year—the winter—and the lack of knowledge of the previous treatment and methods of storage of butter bought on the market, it was decided to use some cod-liver oil also. 8.5% butter fat and 1.5% cod-liver oil were then given the control animals, mixed with the test diets.

These experiments showed no advantage for either method of extraction; the last survivor of a set was sometimes on one diet and sometimes on another, depending upon individual variability. The rats were all taken at four weeks of age.

		Males from 13M	Females from 13M	Males from 80	Average of males from 13M
Diet 97-33	Rat	No. 4915	No. 5135	No. 4919	5 rats
	Wt. in gms.	43	45	43	55
	Survival, days	54	61	62	61
Diet 97-34	Rat	No. 4912	No. 5134	No. 4918	8 rats
	Wt. in gms.	46	45	45	55
	Survival, days	59	60	59	62
Diet 97-35	Rat	No. 4913	No. 5133	No. 4917	5 rats
	Wt. in gms.	45	46	48	53
	Survival, days	62	72	49	57

Since for these animals, at least, the differences noted are practically negligible, it was decided that one method of extraction removed about as much vitamin A as another. As the second method, for preparing Diet 97-34, was the easiest for work requiring continuous preparation of the food, it has since been used in preference to the others. The method is easily kept constant, is economical of alcohol and requires very little attention and manipulation.

Of the rats on the test diets with vitamin A added, one set was run for 140 days. The males grew very well and the females bred. The female on Diet 97-34+vitamin A was the only one to raise her first litter of young—five out of eight being reared. These young were kept on the same diet and made very good growth. The female on Diet 97-33 reared her second litter of seven, but lost her first lot. The female on Diet 97-35 apparently had two litters, both of which were lost, altho none of the first lot were seen.

Since this work shows that Diet 97-34 is adequate in all other respects except vitamin A, a more rigorous procedure was decided upon, for comparison, to see if all the vitamin A had been removed from the meat by the extraction with hot alcohol in the percolator for 12 hours.

A 150 gram portion of the meat residue was used in the percolator and subjected to the following process:

12 hours with acetone	{	A total hot extraction of twelve hours for each of seven successive days.
12 hours with alcohol		
60 hours with alcohol		

It does not seem likely that any measureable amounts of vitamin A could remain in the meat residue after this treatment, if it is removable by fat solvents. The meat residue was used in making Diet 97-70.

Comparative sets were fed, the results of which are given:

<i>Diet 97-70 (7 x 12 hours)</i>				<i>Diet 97-34 (12 hours)</i>			
Rat	Previous diet	Weight gms.	Survival days	Rat	Previous diet	Weight gms.	Survival days
5109 M	13	31	47	5108 M	13	31	40
5110 F	13	34	60	5111 F	13	34	40
5112 F	13	31	50	5113 F	13	31	58
5117 F	13M	35	49	5118 F	13M	34	54
5121 F	13M	42	65	5122 F	13M	42	66
5127 F	90	48	73	5130 F	90	45	67

Since differences between survivals on the diet extracted 7x12 hours and on the diet extracted 12 hours are not significant, or consistently in favor of one of the diets, it may be safely assumed that the 12 hour extraction removes all vitamin A for experimental purposes.

From the experimental proof cited, it may then be said that a vitamin A free diet adequate in all other respects may be easily prepared for experimental work.

IV. EXPERIMENTS WITH YOUNG RATS

EXPERIMENTS WITH YOUNG RATS ON DIETS FREE FROM VITAMIN A

In either curative or preventative experiments for measuring vitamin A, young rats are used. For the planning and interpretation of such experiments it would be necessary to know how young rats could be expected to behave on a diet good in all other respects but free from vitamin A.

Altho the amounts of vitamin A stored in the body of young rats such as are commonly used for this purpose are doubtless smaller than those in the larger animals previously discussed, it is yet a matter of importance. Since in this laboratory litters of young rats are separated and numbered at four weeks of age, they are at that time taken for experimental work. The relation of survival period to initial weight of rats started at four weeks of age, all of which were from families which were receiving the same diet (Diet 13M), is shown in the following table.

Survival Period on Diet Free from Vitamin A

Rats four weeks old from Diet 13M.

Weight at four weeks, grams:	Survival period in days		Females	No. of rat
	Males	No. of rat		
70	75	No. 5002		
61	59	No. 5049		
60	60	No. 4928		
56	69	No. 5008		
55	57	No. 4922		
50	77	No. 5186		
48	55	No. 5187		
47	64	No. 5119.....	54	No. 4934
	73	No. 5066		
46	59	No. 4912		
	61	No. 5169		
45	77	No. 5191.....	60	No. 5134
44	60	No. 5171.....	67	No. 5172
	49	No. 4914		
43	64	No. 5115.....	80	No. 5194
42			66	No. 5122
			65	No. 5052
			54	No. 5173
40			70	No. 5195
39	44	No. 5179		
37			73	No. 5123
34			54	No. 5118
32			47	No. 5184
			38	No. 5174
30			41	No. 5185

There is here observable a slight tendency toward longer survival in the case of the larger rats but the differences attributable to size alone among rats of the same age and dietary history are pronounced only where the weight is much above or below the average. Sex also proves to be of little if any importance in such cases. It is interesting to note that the female of longest survival period (No. 5194) and one of the two males which survived nearly as long (No. 5191) were from the same litter, illustrating the desirability of "litter controls" in comparative experiments wherever practicable. Apparently family and individual variability are larger factors than sex or size among normal rats all of which are 28 or 29 days old and whose dietary histories are identical.

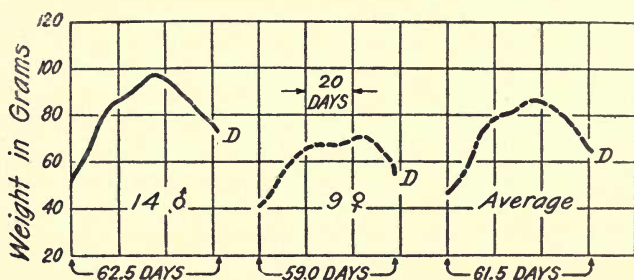


Figure 2. Average weight curves of rats four weeks old when placed on diet free from vitamin A.

There is the suggestion of a slight difference between the sexes in the form of the weight curve during the survival period as shown in the accompanying figure (Fig. 3); but the difference between the average curves for the sexes is so far within the individual variations in either sex that it can only be regarded as suggestive as a possible means of further refinement of the method as data from large numbers of future experiments become available.

For the present the chief use of these survival curves will be to guide the experimenter as to when to expect the downward bend of the curve, indicative of the exhaustion of the body's previous store of vitamin A. On diet 97-34, rats from as good a previous diet as these cannot be expected to start to decline before five weeks on the vitamin A free food. While the average curve for females is lower, it also starts lower. However, it does not bend down before the curve for males; their behavior is so much the same that it seems justifiable to average males and females together in the following discussion of the effect of previous diet.

INFLUENCE OF PREVIOUS DIET ON WEIGHT CURVES OF 4 WEEKS OLD RATS.

For any experiments depending upon the point where the weight curve begins to bend down, as curative experiments for measurement of vitamin A, a careful determination of this sort would have to be made of the young rats used. Once the determination were made, young rats from that same previous diet should be used. Zilva and Miura (16) put animals 50 to 60 grams in weight on the diet free of vitamin A. The majority show little growth during the first 10-15 days, and after that no growth. They say that vigorous rats showing a tendency to grow beyond this time are useless for quantitative work. Doubtless this method has been quite well standardized by these workers, but it could not be duplicated in another laboratory without definite knowledge of the dietary history of the rats used. It is evident, for example, that our Diet 13M rats would all be thrown into the discard as gaining beyond the 15th day.

Comparison and correlation of vitamin A measurements from different laboratories will be almost impossible until more uniformity is reached on the essential points. The variety and non-conformity of results is easily explained by comparisons of current experimental method. The following examples are from laboratories putting out much work:

Steenbock and Sill (15) take rats 3 to 4½ weeks old, at the corresponding weights of 40 to 70 grams.

Zilva and Miura (14) take rats 50 to 60 grams in weight, with no mention of age.

Drummond (1, 2) takes young rats about 50 grams in weight, with no mention of age.

No mention is found of previous dietary history; the young rats are not otherwise standardized or described, except as to sex.

The influence of the previous diet upon the survival period of the four weeks old rat when placed upon a diet adequate in all other respects but free from vitamin A is summarized in the following table giving the average results obtained in this laboratory. The diets here designated as A, B and D are composed entirely of wheat and milk and contain increasing proportions of milk and therefore of vitamin A, in the alphabetical order. The corresponding increase in body store of vitamin A, even at four weeks of age, is plainly apparent in the average survival periods.

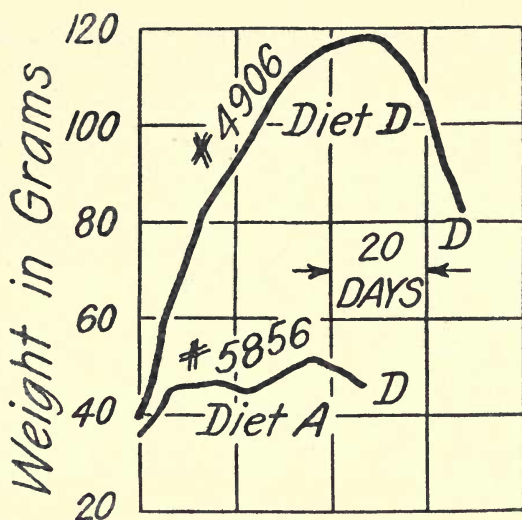


Figure 4. Weight curves of rats from different diets placed at the same age (four weeks) upon the same diet free from vitamin A (Diet 97-34).

Influence of Previous Food on Average Survival Period

Previous Diet	Survival on Diet free from Vitamin A	Number of cases averaged
A (16)	34 days	11 rats (Sherman and Boynton (12))
B (13, 90)	51 days	14 rats M.M.K.
D (80)	64 days	7 rats M.M.K.
13M	61.5 days	23 rats M.M.K.

See also the individual weight curves for diets A and D in Fig. 4.

As previously explained, Diet 13M consisted of a dry food mixture of wheat and milk of the same composition as diets 13 and 90 (Diet B in the table) in addition to which the rats were fed fresh, raw, lean beef at the rate of 60 grams per adult rat per week.

From the above survival periods it is to be inferred that the raw beef adds something to the intake and storage of vitamin A, but not as much as is added by increasing the proportion of milk in the food mixture.

This conclusion is confirmed by experiments in which the same milk and meat were directly tested for vitamin A by feeding separately as sole sources of this vitamin to rats of similar age and size and the same previous dietary history, which had been brought to stationary weight by means of a diet adequate in all other respects but free from vitamin A (diet 97-34). Typical

curves showing the results of feeding 0.2 gram of dry milk and 1.0, 3.0 and 4.0 grams of raw lean beef are shown in the accompanying figure. (Fig. 5).

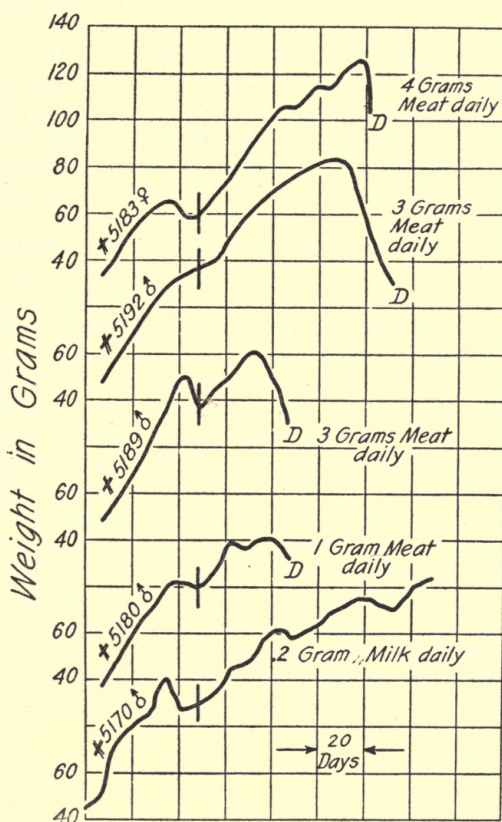


Figure 5. Comparison of meat and milk as sources of vitamin A.

V. SUMMARY AND CONCLUSIONS.

The purpose of this work was to investigate the importance of several factors entering into the problem of the measurement of vitamin A and into the interpretation of data regarding its significance in nutrition.

By means of experiments with rats it is shown that vitamin A is required in the normal nutrition of the adult as well as of the growing organism. That the adult shows less quickly than does the growing animal the effects of the dietary deficiency when placed

on a diet lacking vitamin A is largely due the fact that the adult during such deprivation is able to draw upon the vitamin A which has previously been stored in the body.

A large number of experiments were made with grown as well as growing rats to study the survival periods (on diets lacking vitamin A) with relation to the ages, sizes, and previous histories of the experimental animals. These survival periods serve as measures of the relative amounts of vitamin A contained in the body at the time of placing the animal on the diet devoid of this vitamin. In a series of experiments upon exceptionally well-matched adult animals it was found that this survival period was not appreciably affected by the relative amounts of the mineral elements in the experimental diet devoid of vitamin A, adult maintenance being much less influenced than is growth by the mineral content of the diet. Similarly the protein of white flour, only, while not adequate for satisfactory growth, proved entirely satisfactory in these experiments. Notwithstanding the shortcomings of the diet as regards its protein and mineral content, it was found that the animals died essentially from lack of vitamin A, showing that such survival experiments may be used in studies of this type even with experimental diets which are not adequate for growth in all respects except as to vitamin A. This is important for its bearing both upon the interpretation of experimental data and of human experience with diets deficient in vitamin A, which may also present other deficiencies.

The age at which the storage of vitamin A in the body of an adequately nourished animal reaches a maximum has not been definitely determined and may vary with the diet. For the diets on which we have most data for adult rats the maximum storage seems to have been reached at about four or five months, or approximately at the completion of growth.

Comparing the average results obtained with rats of similar age but different dietary history, it is shown that the richer the diet in vitamin A the greater the amount of this vitamin stored in the body, and hence the longer the survival period when placed upon a diet devoid of vitamin A. Such comparisons of survival periods of rats from different diets but of the same age can thus be made a method for the study of the vitamin A content of the respective diets on which the rats were reared.

For the more usual method of measuring vitamin A, which consists in feeding experiments with young growing rats, it is important that a diet adequate for growth in all other respects and entirely devoid of vitamin A should be used. Such a diet is here described. Its freedom from vitamin A is shown from the fact that much longer extraction for removal of this vitamin has no further effect; and its adequacy in other respects is shown in that when vitamin A was added the diet was proven adequate not only for growth at a fully normal rate but also for reproduction.

In order to minimize discrepancies due to previous storage, the young rats here used were placed upon the diet devoid of vitamin A when they were four weeks old. Even at this age the effect of the previous diet of the family upon the store of vitamin in the body of the young rat, and therefore upon the weight curve and survival period, proved to be a very much more important factor than was previously known.

The bearing of these findings upon the standardization of methods for the determination of vitamin A is discussed, and the application of the method to the measurement of relative amounts of vitamin A in different foods is illustrated.

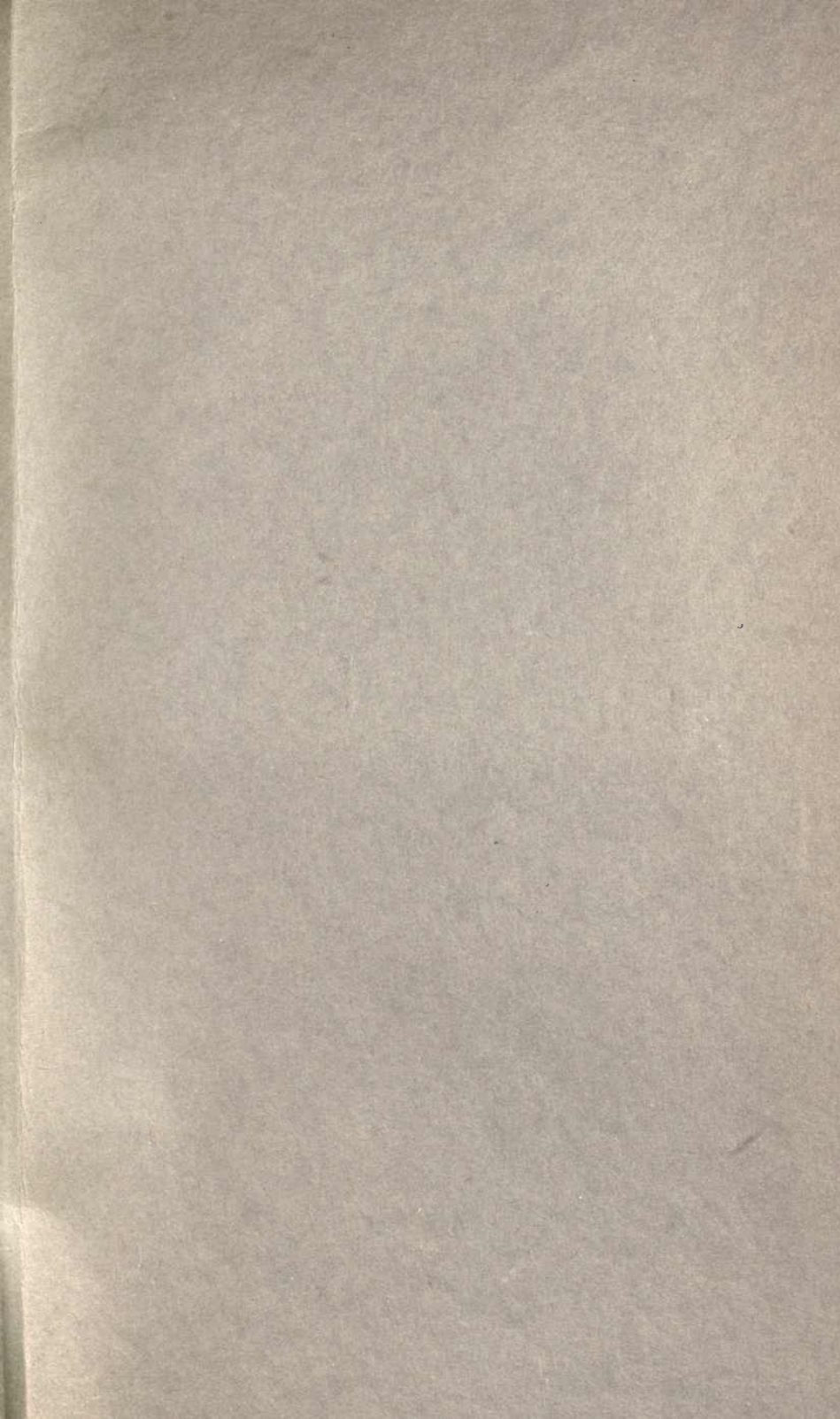
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VITA

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